STUDIES ON THE ACROSOME. IV. THE ACROSOME REACTION IN SOME BIVALVE SPERMATOZOA[1]

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An earlier study (Dan, 1952) has demonstrated that the spermatozoa of several sea urchin species respond to certain stimuli by a characteristic reaction of the acrosome, in which the membrane covering its anteriormost part appears to break down, exposing the underlying substance. Part of this exposed material disperses almost immediately, leaving a short filamentous structure which remains essentially unchanged thereafter. This reaction is induced by egg-water of the same species, by alkaline sea water (pH 9.2) and by contact of the spermatozoa with such surfaces as glass and collodion.

In starfish spermatozoa, an acrosome reaction of a rather different sort takes place (Dan, 1954). The nearly spherical heads of these spermatozoa are flattened anteriorly, and the acrosome is imbedded in the nuclear region. If the spermatozoa are suspended in a 0.5% solution of crystallized egg albumin in sea water (as adjuvant), and then mixed with egg-water of the same species, a very long (ca. 25 μ) straight filament is extruded from the center of the acrosome surface and simultaneously certain changes take place in the interrelations of the head, middle piece and tail. The diameter of this filament is somewhat less than that of the axial filament of the tail, and it possesses considerable rigidity. Like the much shorter filaments of sea urchin sperm, these structures are relatively durable in sea water.

It has been found that the spermatozoa of several representative bivalve species undergo a similar reaction. These animals were chosen as objects for the study of the acrosome reaction because in many species of this class fertilization takes place externally and can be accomplished experimentally. Some of the species available in the vicinity of Misaki have been observed in their respective breeding seasons; the sampling includes representatives from three of the five orders, and eight families.

These animals generally have spermatozoa in which it is easy to identify the acrosome with the high power of the phase contrast microscope, and to differentiate it from the other components of the sperm head. In some of the species examined in this study, the stimulus of contact, alone or in the presence of egg-water, causes the disappearance of the original structure, and there appears in its place a filament of about the same diameter as that of the starfish filament. In the other species, the same sort of reaction was observed to have taken place in (supernumerary) spermatozoa in the vicinity of the egg surface at fertilization.

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This complete disappearance of the acrosome in *Mytilus edulis* was reported by Kupelwieser in 1909 and by Meves in 1915. Both workers found that the sperm head just inside the cytoplasm of eggs immediately after fertilization lacked any sign of the "perforatorium." Meves comments (p. 53): "Merkwürdig ist, dass das Perforatorium nicht nur bei den völlig aufgenommenen Köpfen, sondern auch schon bei den noch im Eintritt begriffenen spurlos geschwunden ist; sein Substanz muss also sehr rasch auflösen."

This paper will deal with the morphological aspects of the acrosome reaction in twelve species of bivalve molluscs.

Materials and Methods

Observations were made on the spermatozoa of the following Pelecypoda:

Mytilidae
- *Mytilus edulis* Linné
- *Lithophaga curta* (Lischke)

Spondylidae
- *Spondylus cruentus* Lischke

Ostreidae
- *Crassostrea* (= *Ostrea*) *echinata* (Quoy et Gaimard)
- *Crassostrea* (= *Ostrea*) *nippona* (Seki)
- *Crassostrea* (= *Ostrea*) *gigas* (Thunberg)

Trapeziidae
- *Trapezium sublaevigatum* (Lamarck)

Chamidae
- *Chama retroversa* Lischke

Petricolidae
- *Petricola japonica* Dunker

Mactridae
- *Mactra veneriformis* Reeve
- *Mactra sulcataria* Reeve

Pholadidae
- *Zirfaea subconstricta* (Yokoyama)

In this survey, attention was directed to those species in which artificial fertilization was known to be possible, and in every case, the utmost effort was made to induce spawning, because it was early found that the reacting capacity of spawned spermatozoa is generally much greater than that of sperm taken from excised testes. Methods for inducing spawning of bivalve molluscs have been reported by various workers; they include exposure of the animals to sperm- or egg-charged sea water (Galtsoff, 1938, 1940; Wada, 1954), electrical stimulation (Iwata, 1949), raising of water temperature (Wada, 1936; Galtsoff, 1938, 1940). Variations and combinations of these methods were often found effective; in some of the species reactive spermatozoa were obtained by simple excision of the testes, or by excision following some kind of stimulation.

The animals were used as soon after collection as possible, and were always kept in running water in the laboratory. When increased temperature was used

to induce spawning, the incoming sea water was run through a glass coil immersed in a vessel of water heated by an electric heating unit; the temperature of the water surrounding the animals was regulated to 25–28° C. by adjusting the rate of flow.

Sperm- and egg-suspensions for inducing spawning were prepared by adding the gametes from an opened male or female to a suitable volume of sea water. These suspensions were added to the sea water containing the animals, sometimes alternately, and often following an extended period of warming. In some cases animals were induced to spawn by the second or third such stimulus, after having been continuously at the high temperature for several hours.

Electrical stimulation was used particularly in the case of *Mytilus*, following the method of Iwata. The shells of the mollusc are held apart by a wooden wedge about 5 mm. thick, and a small roll of absorbent cotton moistened with sea water is partially inserted into the space between the shells near the narrow end, so that it comes into contact with the tissues. The animal is then supported over a vessel filled wih sea water so that the wide end is immersed in the sea water. A variable resistance is interposed in the ordinary (50 cycle) alternating current and adjusted so that about 20 volts passes through the completed circuit. One Ag-AgCl electrode is dipped into the sea water, while the other is brought into contact with the cotton wad for 15 seconds. The wedge is then removed and the stimulated animals placed in sea water in separate containers until they spawn.

Egg-water for inducing the acrosome reaction was prepared by the method used for echinoderms. Unfertilized eggs are suspended in a small volume of sea water and left standing, usually for 30 minutes or more. The supernatant of this suspension is filtered before use.

As in the case of starfish spermatozoa, the acrosome filaments of bivalve sperm are not satisfactorily preserved in mass suspensions by formalin fixation. With this method, even with very dilute formalin, the material of the filament, when it can be detected at all, is in the form of a rounded mass on the anterior part of the sperm head. It is possible, however, to obtain a rather unsatisfactory fixation with formalin of filaments which are stuck on a glass, collodion or formvar surface. With osmic vapor, the filaments are well preserved, but the sperm heads are distorted by swelling, and often break down entirely on subsequent washing and drying.

The most satisfactory observations were made by placing a small amount of living sperm suspension on a coverglass which was inverted on a slide and observed with the phase contrast immersion objective, using anisol ($C_6H_5 \cdot O \cdot CH_3$—refractive index = 1.515) as the immersion fluid. (Since this substance has a low viscosity, its use instead of cedar oil obviates the necessity for fixing the coverglass in place.) The spermatozoa which have reacted on contact with the coverglass become attached to its under surface, where they are most successfully observed. Unfortunately, at this magnification (ca. 1000 ×) the focal depth is so shallow that it will not include both the filament on the underside of the coverglass and the outline of the sperm head about 1.5 μ below it. For this reason photography was not practicable, and records had to be made in the form of camera lucida drawings or free-hand sketches.

Results

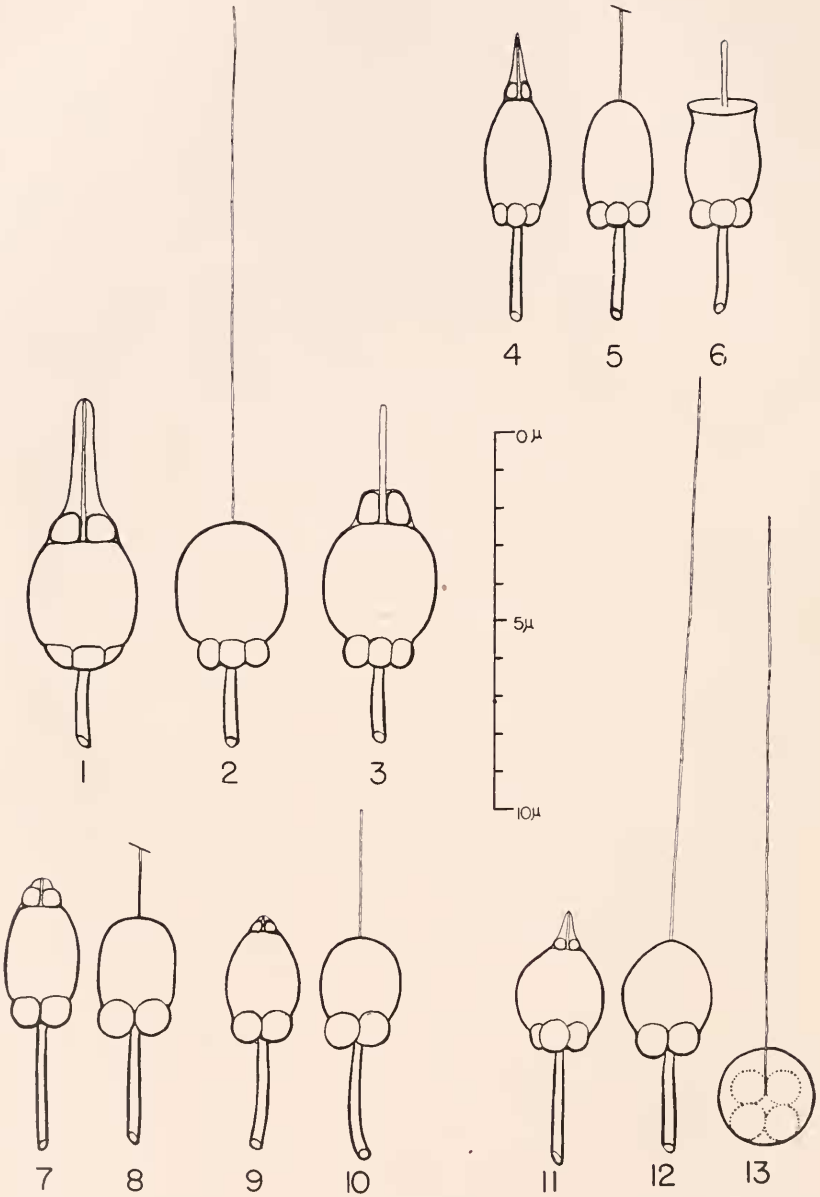
The spermatozoa of all these species resemble each other more or less closely in such fundamental characteristics as the shape of the nuclear part of the head, and the fact that the middle piece takes the form of four or five small spheroidal bodies arranged in a ring around the insertion of the flagellum. The acrosomes are very different in size, but always appear as cones affixed to the anterior part of the nucleus. In some cases the cones are so low that it is barely possible to establish their existence, but in the species with conspicuous acrosomes there is clearly visible an axial differentiation, which might represent either a tubular passage through the center of the acrosomal cone or a fibrillar structure.

When the acrosome is large enough so that its substance can be distinguished from the surrounding membrane, it appears (with phase contrast, dark contrast) to be filled with a rather strongly refringent hyaline material. In the largest acrosomes (e.g., that of *Mytilus edulis*) it is possible to see a clearly differentiated basal part which has considerable structural rigidity, and there is evidence that such a structure is also present in small acrosomes. In 1% OsO_4-sea water, this structure and the spheres making up the middle piece are not blackened after 30 minutes' fixation.

In all the twelve species examined, the reaction of the spermatozoa to egg-water, or to contact with an egg or other surface such as glass or collodion, results in a complete disappearance of the original acrosome. In its place there appears a slender, rigid filament, extending directly forward from the center of the area previously covered by the acrosome. It is difficult to estimate the diameter of these filaments, since it is at the limit of resolution of the optical system when they are observed in the living state with the light microscope, while measurements of electron micrographs involve several serious sources of error, such as possible shrinkage on fixation, and undoubted distortion (flattening and shrinkage) during mounting and desiccation in preparation for observation *in vacuo*. Both in the living state and in electron micrographs the acrosome filament appears to be of approximately the same diameter as the axial filament of the sperm tail.

In the course of these observations it became evident that the reacted (supernumerary) spermatozoa which are seen around an egg after fertilization do not, as was first assumed, represent individuals which have responded to the stimulus of substances from the egg at a little distance from it and have been prevented, by some monospermy-insuring mechanism, from penetrating its surface. There is evidence that the spermatozoa in these molluscan species react only after they have established contact with the vitelline membrane, the acrosome filaments of all the reacting spermatozoa first extending into the egg cytoplasm, and later all but the fertilizing spermatozoan being expelled. Since this process apparently involves a shortening of the acrosome filament, the true filament length of these supernumerary sperm may be considerably greater than that observable by the methods hitherto used. This question will be discussed in a later paper.

It is a common characteristic of all the observed species that reacted spermatozoa show a reduction in activity, together with a "loosening" of the head structure, similar to that which occurs in the starfish sperm. This is most clearly observed in a changed appearance of the spherules making up the middle piece. In the spermatozoan before reaction, these are usually much compressed so that it is often



FIGURES 1–29. Sketches of some bivalve spermatozoa, showing morphological changes resulting from the acrosome reaction.

FIGURE 1. *Mytilus edulis* spermatozoan in sea water.
FIGURE 2. *Mytilus* sperm after reaction induced by egg-water.
FIGURE 3. *Mytilus,* partial reaction.
FIGURE 4. *Petricola japonica* spermatozoan before reaction.

difficult to determine their number, and their outline is often more or less continuous with the curve of the sperm head. After the reaction, on the other hand, they are more conspicuous as separate spheres, as though a tight enveloping membrane had been relaxed (*cf.* Figs. 1 and 2, 4 and 5, etc.). There is also a tendency, in elongated spermatozoa, for the anterior part of the nucleus, after the breakdown of the acrosome, to lose the truncated cone shape and become more nearly spherical (Figs. 7 and 8, 17 and 18).

In the following section, the methods used for obtaining gametes and the characteristics of the spermatozoa and their acrosome reaction are reported for each species. The approximate breeding season at Misaki is indicated in parenthesis.

Mytilus edulis (Autumn and winter)

Spawning was induced by electrical stimulation. During most of the breeding season these animals begin to shed eggs or sperm about 40 minutes after being stimulated at 13–15° C., and within 30 minutes at 18–20° C. Shedding males were usually removed to dry Syracuse watch glasses, so that the sperm could be obtained with a minimum of dilution.

The large *Mytilus* spermatozoan has a strikingly prominent, pointed acrosome (Figs. 1, 30), which is 3.5 to 4.5 μ in length, somewhat more than the combined length of the nucleus and middle piece. Through the center of this extended cone there is a clearly visible axial structure, and at its base, a differentiated region which appears in living spermatozoa as a thick ring forming the base of the acrosome.

These spermatozoa undergo the acrosome reaction in a relatively high percentage if spawned spermatozoa are mixed with egg-water. In this reaction (Figs. 2, 31), the whole acrosome breaks down, and in its place there is extruded a very slender filament about three times the length of the original acrosome. When the fertilization process is observed with phase contrast high power, such a filament can be seen, extending between the heads of supernumerary spermatozoa and the egg surface. The fertilizing spermatozoan is always closely against the vitelline membrane by the time it can be brought into focus.

It has so far not been possible to observe the acrosome in the actual process of breaking down under the stimulus of egg-water, because of the intense activity of the spermatozoa. However, when they are suspended in 0.05% merthiolate-sea water, the spermatozoa are immobilized at once, and the breakdown of the acrosome follows, but not immediately or simultaneously, so that it is possible to observe the process under these conditions. The acrosome breaks down progressively from

FIGURE 5. Reacted *Petricola* spermatozoan as found in vicinity of egg immediately after fertilization (supernumerary sperm).

FIGURE 6. Partial reaction in *Petricola* sperm.

FIGURE 7. *Mactra sulcataria* spermatozoan.

FIGURE 8. *M. sulcataria* supernumerary spermatozoan.

FIGURE 9. *Mactra veneriformis* spermatozoan.

FIGURE 10. *M. veneriformis* spermatozoan after acrosome reaction induced by egg-water.

FIGURE 11. *Spondylus cruentus* spermatozoan.

FIGURE 12. *Spondylus* sperm reacted in egg-water (side view).

FIGURE 13. Reacted *Spondylus* spermatozoan as found affixed to under side of coverglass by adhesive anterior surface.

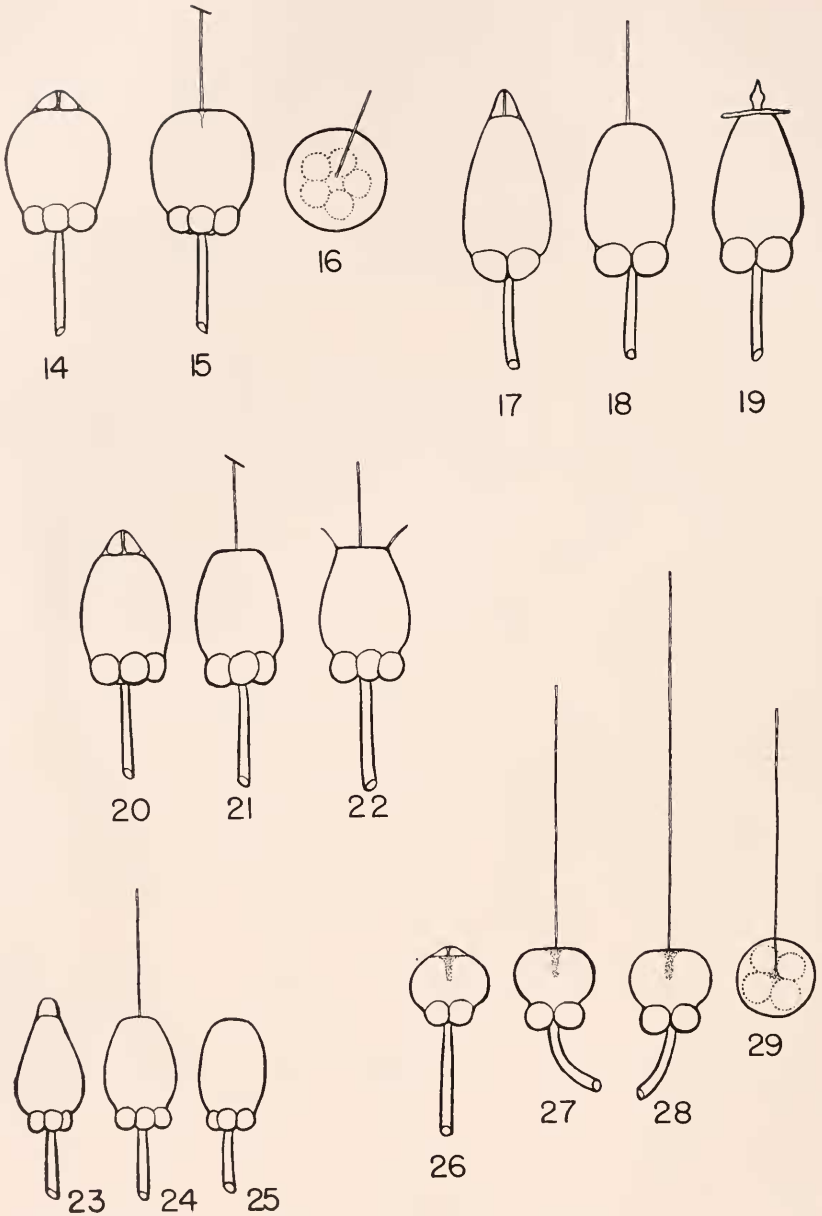


FIGURE 14. *Zirfaea subconstricta* spermatozoan.
FIGURE 15. Supernumerary *Zirfaea* sperm in side view.
FIGURE 16. Supernumerary *Zirfaea* sperm stuck to coverglass by adhesive anterior surface.
FIGURE 17. *Lithophaga curta* spermatozoan.
FIGURE 18. Reacted *Lithophaga* sperm.
FIGURE 19. Partial reaction of *Lithophaga* spermatozoan.
FIGURE 20. *Chama retroversa* spermatozoan.

the tip, within a fraction of a second, leaving no trace of the original structure except a small remnant at the base. The filament in these cases is shorter and stouter than normal. There is no evidence that the effect of merthiolate is identical with that of substances from the egg—this observation is offered simply as the only presently available information as to the manner in which the process of acrosome breakdown may proceed.

Some further inferences can be tentatively drawn from observation of spermatozoa which have undergone what has been provisionally called "partial reaction" (Fig. 3, *cf.*, Figs. 6, 19). In such spermatozoa, either because of their sub-normal condition or as the result of a sub-optimal stimulus, the reaction has apparently stopped with the breakdown of the distal part of the acrosome, leaving a basal collar-like structure. Such spermatozoa always show a filament which is about the length of the original acrosome.

Lithophaga curta (Late spring)

Males spawned on the third addition of egg-suspension to animals in warm (28° C.) running sea water. The spermatozoa thus discharged were intensely active, and their acrosomes reacted on contact with the coverglass (Figs. 17, 18).

In this species there were many cases of "partial breakdown," in which some material, presumably the covering of the acrosome, remained attached to the anterior part of the head; and a poorly defined pointed rod, corresponding to the axial structure of the intact acrosome, could be seen still in its original position (Fig. 19).

Spondylus cruenta (Summer)

Active spermatozoa were obtained by extirpation of the testes, and these readily underwent the acrosome reaction on addition of egg-water prepared by removing the supernatant from a 5% suspension of eggs after 10 minutes (Figs. 11, 12). Reacted sperm showed a strong tendency to adhere to the coverglass by the anterior surface of the head, with the acrosome filament bent perpendicular to its normal position (Fig. 13). This behavior, which indicates that the breakdown of the acrosome leaves the sperm head surface locally sticky, is also found in the spermatozoa of the oysters and *Zirfaea*. In *Spondylus* the sperm head is slightly asymmetrical bilaterally, and the figure presented by the adhering spermatozoan is correspondingly somewhat off-center.

FIGURE 21. Supernumerary *Chama* spermatozoan.

FIGURE 22. Reacted *Chama* spermatozoan, acrosome covering incompletely broken down.

FIGURE 23. *Trapezium sublaevigatum* spermatozoan.

FIGURE 24. Reacted *Trapezium* sperm.

FIGURE 25. *Trapezium* spermatozoan in which acrosome has broken down but no filament is apparent.

FIGURE 26. Spermatozoan of *Crassostrea echinata, C. nippona, C. gigas.*

FIGURE 27. Spermatozoan of *C. echinata* or *C. gigas*, reacted on contact with glass surface in presence of egg-water (side view).

FIGURE 28. Reacted sperm of *C. nippona*, from side.

FIGURE 29. Anterior view of reacted *C. echinata* spermatozoan attached to coverglass by adhesive surface.

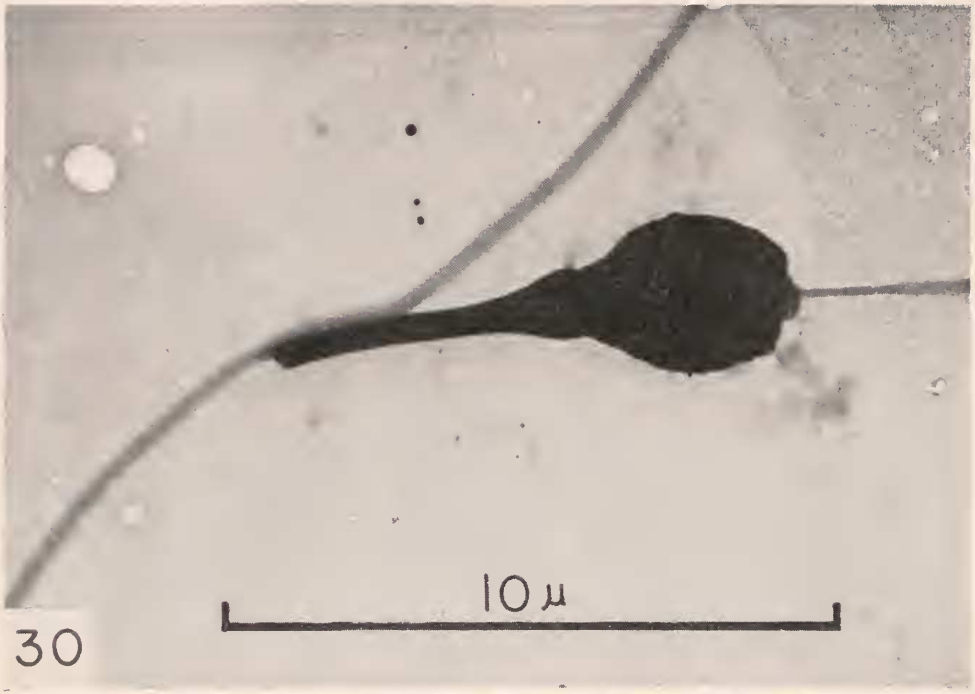


FIGURE 30. Electron micrograph of *Mytilus edulis* spermatozoan fixed with formalin in sea water.

FIGURE 31. Reacted *Mytilus* spermatozoan (formalin fixation).

Fertilization with the gametes obtained in this way was only possible when the pH of the sea water was increased by the addition of NH_4OH to a final concentration of 1.0×10^{-3} N. Filaments were observed connecting the supernumerary sperm with the egg surface.

Crassostrea echinata (Summer)
C. nippona
C. gigas

Spawning was induced by adding egg-suspensions to the tanks containing animals in warm (28° C.) running sea water. Fairly reactive gametes can also be obtained by simply cutting open the animals. It was found that the best method for obtaining a dense suspension of maximally reactive sperm is to cut open a male which has begun to spawn in response to the warming-plus-egg-water stimulus.

The spermatozoa of these three species are indistinguishable both before and after the acrosome reaction, except that the filaments in *C. nippona* are longer than those of the other two species. The intact acrosome is a small, low cone, which is affixed to the anterior part of the nearly spherical head (Figs. 26, 32). Under the center of the acrosome there extends radially inward a gray-appearing (with dark phase-contrast) structure or region not sharply differentiated from the surrounding nuclear material; this is even more clearly visible after the acrosome has reacted, and the acrosome filament seems to extend directly from its center (Fig. 27).

In these species the shape change accompanying the acrosome reaction tends to flatten the anterior surface of the sperm head, which becomes extremely adhesive as the result of the acrosome breakdown (see also *Spondylus*), and sticks to the glass by this surface (Fig. 29).

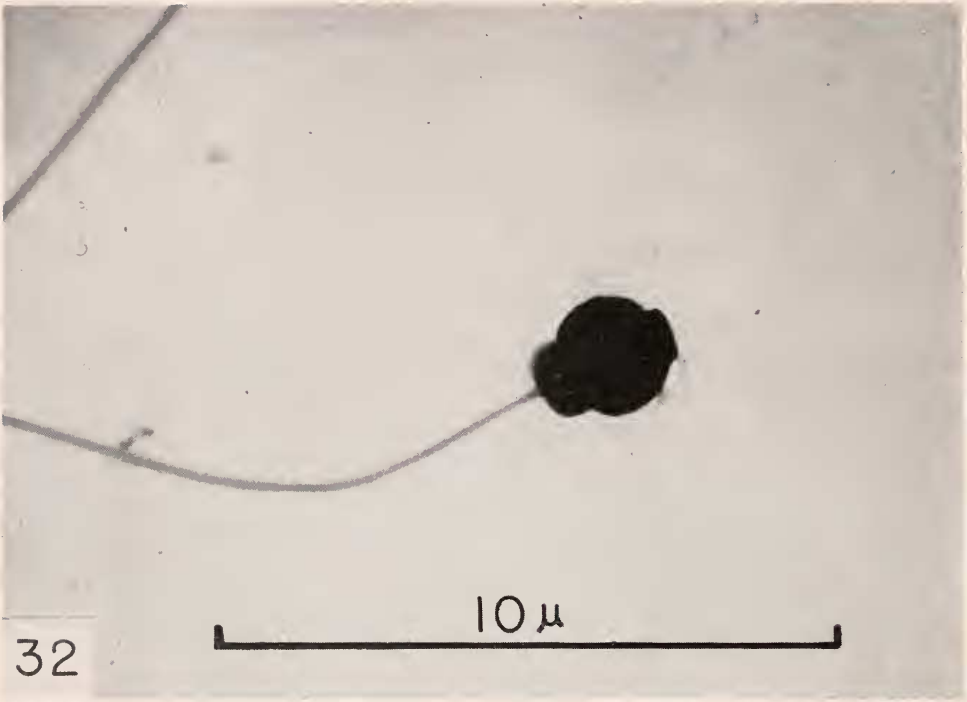
Intermediate stages in the acrosome reaction indicate that the extrusion of the filament occurs simultaneously with the breakdown of the original acrosome surface. It is probable that Figure 33 represents a case in which the normal process was stopped by fixation.

In order to obtain a high percentage of reacted acrosomes, the spermatozoa of these species must be brought into contact with an egg, or with some other surface in the presence of egg-water. It makes no difference whether the eggs are unfertilized, fertilized or even cleaved, and if the suspension is stirred sufficiently, the presence of a few eggs will induce reaction of the acrosomes in most individuals of a relatively dense sperm suspension.

Trapezium sublaevigatum (Late summer)

Gametes were obtained by cutting open the animals. The spermatozoa were intensely active in sea water suspension, but the eggs were very fragile and easily broken in handling.

In the vicinity of the eggs, the spermatozoan acrosomes broke down, and many sperm were observed attached to the vitelline membranes by filaments about 3 μ in length (Figs. 23, 24). In other cases the acrosomes were gone but no filaments were in evidence (Fig. 25). Such spermatozoa were often attached



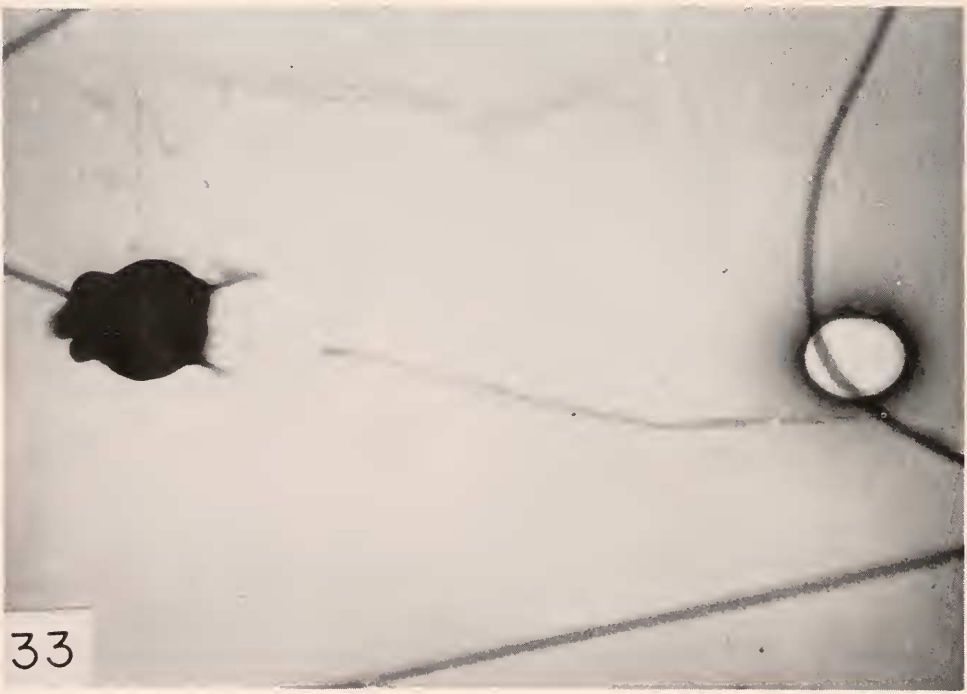


FIGURE 32. Spermatozoan of *Crassostrea gigas* in sea water. Formalin fixation has somewhat distorted shape of acrosome.

FIGURE 33. *C. gigas* spermatozoan reacted in response to contact with collodion membrane

obliquely to the coverglass by the anterior part of the head. In these sperm, also, the breakdown of the acrosome apparently leaves the underlying surface sticky, although not as markedly so as in the oysters.

Chama retroversa (Summer)

Sperm and eggs were obtained from opened animals. The spermatozoa showed vigorous activity on suspension in sea water, and fertilization took place readily in normal sea water. In this species also, the fertilizing spermatozoan was already close against the vitelline membrane at the earliest observation, but supernumerary sperm were found in the jelly layer with extruded filaments (Fig. 21). As suggested above, the length of the acrosome filament in such cases is doubtful.

The blunt acrosome in this species (Fig. 20) consists of an axial, differentiated structure surrounded by refringent substance. Not infrequently individuals are found in which the covering membrane of the acrosome has apparently failed to break down completely (Fig. 22).

Petricola japonica (Late spring and early summer)

These animals were induced to spawn by exposure to warm running sea water. Spermatozoa removed from opened animals were quiescent on being suspended in sea water, but became intensely active after a short time. These spermatozoa have a relatively long (ca. 1.7 μ), sharply pointed acrosome (Fig. 4) with a well-defined axial structure. In some cases the acrosome may break down, leaving a filament which is just the length of the original acrosome, and some other material which persists around the base of this filament (Fig. 6). In the complete reaction, as seen in supernumerary spermatozoa (Fig. 5), the acrosome breaks down entirely.

Mactra veneriformis (Late spring and early autumn)

Reactive gametes were secured by opening the animals. The eggs remain fertilizable for several hours if they are left in the body fluid of the clams, but the germinal vesicle breaks down when the eggs are introduced into sea water, and the eggs can then no longer be fertilized. Practically 100% fertilization was obtained with this species in 80% sea water.

The spermatozoa have small acrosomes in which an axial differentiation is just visible (Fig. 9). The heads of completely reacted spermatozoa are smoothly rounded, with no trace of the acrosome except the filament (Fig. 10), although partially reacted sperm are frequently encountered. On supernumerary sperm the filament is always very short (1 to 1.5 μ), but in a few cases filaments of between 3 and 3.5 μ were produced on reaction with strong egg-water.

M. sulcataria (Spring to early autumn)

Spawning was induced by warming to 25° C. Reactive gametes were also obtained by cutting open the animals.

The spermatozoa of this species are similar in their proportions to those of *M. veneriformis*, but larger (Fig. 7). The acrosomal differentiation into the same

three components as those of the *Mytilus* spermatozoan is clearly observable; the basal structure includes the greater part of the refringent acrosome substance, but a smaller amount lies separate from and distal to this.

The acrosome reaction was observed in supernumerary sperm only. These showed a short filament and the sperm head smoothly rounded anteriorly, as in *M. veneriformis* under the same conditions (Fig. 8).

Zirfaea subconstricta (Late spring)

These animals spawned in response to the combined warming (25° C.) and egg-suspension treatment. On insemination, nearly all the spermatozoa in the vicinity of an egg are found to have undergone the acrosome reaction (Figs. 14, 15), some remaining attached to the vitelline membrane by the relatively short (ca. 2 μ) acrosome filament, and others adhering to the coverglass by the distal part of the head (Fig. 16), indicating that the breakdown of the acrosome leaves the surface under it sticky (as in *Spondylus* and *Crassostrea*).

Discussion

In the first study of this series, the term "acrosome breakdown" was used tentatively, even hesitantly, to describe a phenomenon which takes place in sea urchin spermatozoa on such a small scale that vital observation is virtually impossible. No structure is visible in the intact acrosome, the reaction itself cannot be observed in the intensely active spermatozoa, and all judgments concerning the steps in the process must be based on successively fixed preparations. These clearly show that some of the acrosome substance is lost from the anteriormost tip of the sperm head, leaving a slender filament, less than one micron in length. Whether this filament is projected beyond the original length of the acrosome, or is simply exposed by the dispersal of the surrounding substance, could not be determined. The fate of the acrosome membrane is also rather uncertain. Apparently the anterior part at least undergoes some sort of autolysis which stops before it quite reaches the base of the acrosome.

So far as the morphology of the structures involved in the acrosome reaction is concerned, the bivalve molluscs provide much more favorable material for study, since in some species (*e.g.*, *Mytilus edulis*) the acrosome alone is longer than the combined head and middle piece of sea urchin spermatozoa, and considerable internal differentiation is easily visible in the living cells. In this group, moreover, exposure to the proper stimulus definitely results in a complete breakdown and dispersal of the acrosome, while the filament is clearly projected well beyond its original dimensions.

Among the species examined, the general structure of the head and acrosome is much the same throughout the group, although there is a considerable size range. Since the reaction process occurs similarly in all the species, it seems safe to generalize on the basis of observations made on the larger forms.

The differentiation of the acrosome into three regions has been described as it appears in the living *Mytilus* spermatozoan; this differentiation can be followed in the descending size scale through *Petricola* and *Spondylus* to *Mactra*. In four other genera—*Lithophaga*, *Chama*, *Zirfaea* and *Crassostrea*—only two regions can be seen, consisting of an axial structure passing through the hyaline acrosome

substance; and in the very small acrosome of *Trapezium* no clear differentiation can be observed.[3]

Evidence which will be reported separately indicates that in *Mytilus*, the breakdown of the acrosome releases a substance which has a strongly lytic effect on the egg membrane. Since both the basal and distal parts of the acrosome break down together, it is impossible to localize the lysin in either of them on the basis of cases in which the reaction has occurred normally. However, the observation reported above of "partial reaction," in which the intact basal structure is associated with failure of filament extrusion, suggests that the mechanism which ejects the filament is located in this basal portion, and consequently, that the lysin is contained in the distal part of the acrosome.

"Partial reaction" in the spermatozoa of other species (*e.g.*, *Petricola* and *Lithophaga*) is also characterized by the persistence of an axial structure which is not longer than the intact acrosome, and is greater in diameter than the normally extruded filament. On the basis of these various observations, it is suggested that the axial structure consists of a tubular sheath, possibly enclosing some part or precursor of the filament.

In support of the conjecture that the basal part of the acrosome has the function of ejecting the filament, it would be gratifying to find that the length of the filament was correlated with the size of this basal structure in the other genera studied. This, however, is not the case. The acrosome of *Spondylus* is much smaller than that of *Mytilus* (*cf.* Figs. 1 and 11), but the *Spondylus* filament, measuring 15 μ on an average, is longer than the *Mytilus* filament. *Crassostrea*, also, has a still smaller acrosome but a relatively long filament. Such lack of correlation between the size of the basal part of the acrosome and the length of the filament does not preclude the possibility that this structure is concerned with the extrusion of the acrosome filament in bivalve molluscs, but it does emphasize the necessity for further investigation. Some other method besides phase contrast microscopic observation must also be used to determine whether the apparent absence of differentiation of the acrosome substance in the second group (*Lithophaga*, etc.) indicates a marked reduction in the amount of lysin, as compared with the first group, or a much smaller filament-ejecting apparatus.

This survey of the acrosome reaction in the bivalves, then, complements the echinoderm studies by confirming the generality of occurrence of both the acrosome filament and the substance believed to be the egg-membrane lysin, and by providing, within the limits of the common phenomenon, a scale of cases ranging from those in which the filament is more conspicuous to those in which the lysin seems more important. Until, however, this substance can be shown to be effective in dissolving the egg-membrane in a number of species, an inquiry into the possible reasons for its presence in larger or smaller amounts is premature.

Moreover, until more is known about the role of the acrosome filament in the fertilization process, it is difficult to identify the factors which determine its length. There does not seem to be a simple taxonomic relation involved, since long and short filaments are found in closely related genera (*e.g.*, *Mytilus* and *Lithophaga*). The idea that the filament serves to establish contact with the egg surface across

[3] This may well be due to a failure of phase contrast resolution, since the acrosome is less than 0.5 μ in inside diameter, and its substance is highly refractive.

a jelly layer which is impenetrable to the intact spermatozoan was suggested in the case of the starfish, but is entirely inapplicable to molluscan species having jellyless eggs, such as the oysters. Moreover, in other species, the intact spermatozoan has no difficulty in penetrating the jelly layer when one is present.

Undoubtedly the structural and chemical nature of the outermost layer of the unfertilized egg in each species is a most important factor in determining the characteristics of the acrosome which is charged with the task of breaching that particular barrier. Electron micrographs of the unfertilized *Arbacia* egg (McCulloch, 1952) show it to have an extremely thin vitelline membrane, which would be expected to yield quickly to the action of a small amount of a specific lysin. The oyster egg, on the other hand, is surrounded by a relatively thick, though apparently not tough, layer, to the outside of which the fertilizing spermatozoan remains so closely fixed as to seem fused with it for from two to four minutes, before slowly sinking through it into the egg.

In conclusion, it should be pointed out that this survey was undertaken primarily with the aim of extending the generality of the acrosome reaction, and makes no pretense of adequately sampling the Pelecypoda in a systematic sense. A few species were found in which the spermatozoa could not be caused to exhibit any reaction of the acrosome similar to that reported here, but in every case the basic morphology of such spermatozoa differs considerably from the fundamental plan characteristic of these twelve species. The spermatozoa of *Venerupis semidecussata,* for example, has a cone-shaped head like that of sea urchin sperm, with a long, slightly curved, sword-like process, greater in diameter than the sperm tail, in the position of the acrosome. There is some evidence that a filament contained within this process is exposed at the time of fertilization. The sperm head of *Cardita leana,* on the other hand, is extremely slender throughout its length, with a very small, sharply pointed acrosome in which no reaction of any sort has yet been detected.

There is every possibility that further work with these and other species will bring to light variations in the general pattern of acrosome structure in this molluscan group. Such studies must be correlated with an investigation of the finer structure of the surface layers in the unfertilized eggs before extensive generalizations can be made concerning the mechanism of sperm entrance in these forms.

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Summary

1. In twelve species of bivalve molluscs, it has been found that the spermatozoa undergo a reaction of the acrosome similar to that found among echinoderms. In all the species the spermatozoa react thus to the presence of unfertilized eggs; in some species egg-water and contact with glass surfaces are effective.

2. This reaction is characterized by the complete disappearance of the acrosome and the extrusion of a slender filament, the length of which varies considerably among the members of the group.

3. In the large *Mytilus* acrosome, three differentiated regions can be distinguished, consisting of a basal structure which seems to be concerned with the extrusion of the filament; a distal region containing what is believed to be an egg-membrane lysin; and an axial structure which appears to be a tubular sheath, possibly surrounding a precursor of the filament. The same regions can be seen in the spermatozoa of *Petricola*, *Spondylus* and *Mactra*. In the smaller acrosomes of *Lithophaga*, *Chama*, *Zirfaea* and *Crassostrea*, only two regions can be distinguished, and the acrosome of *Trapezium* is too small to show any clear differentiation.

LITERATURE CITED

Dan, J. C., 1952. Studies on the acrosome. I. Reaction to egg-water and other stimuli. *Biol. Bull.*, **103**: 54–66.

Dan, J. C., 1954. Studies on the acrosome. II. Acrosome reaction in starfish spermatozoa. *Biol. Bull.*, **107**: 203–218.

Galtsoff, P. S., 1938. Physiology of reproduction of *Ostrea virginica*. II. Stimulation of spawning in the female oyster. *Biol. Bull.*, **75**: 286–307.

Galtsoff, P. S., 1940. Physiology of reproduction of *Ostrea virginica*. III. Stimulation of spawning in the male oyster. *Biol. Bull.*, **78**: 117–135.

Iwata, K. S., 1949. Spawning of *Mytilus edulis*. II. Discharge by electrical stimulation. *Bull. Japan. Soc. Sci. Fish.*, **15**: 443–446. (In Japanese)

Kupelwieser, H., 1909. Entwicklungserregung bei Seeigeleiern durch Molluskensperma. *Arch. f. Entw.*, **27**: 434–462.

McCulloch, D., 1952. Note on the origin of the cortical granules in *Arbacia punctulata* eggs. *Exp. Cell Res.*, **3**: 605–607.

Meves, F., 1915. Ueber den Befruchtungsvorgang bei der Miesmuschel (*Mytilus edulis* L.). *Arch. mikr. Anat.*, **78**: Abt. **2**: 47–62.

Wada, S. K., 1936. Thermal stimulation of spawning in the Japanese pearl oyster. *Suisan Gakkwai Ho*, **7**: 131–133. (In Japanese)

Wada, S. K., 1954. Spawning in the tridacnid clams. *Jap. J. Zool.*, **11**: 273–285.